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## RAW SEQUENCE LISTING

DATE: 02/28/2002

PATENT APPLICATION: US/09/876,796B

TIME: 12:22:24

Input Set : A:\Rb125seq.txt

Output Set: N:\CRF3\02282002\I876796B.raw

3 <110> APPLICANT: Horwath, K. L. , Easton, C. M. , and Myers, K. L.  
 5 <120> TITLE OF INVENTION: Nucleic Acid Sequences Encoding Type III Tenebrio  
 6 Antifreeze Proteins and Method for Assaying Activity.  
 8 <130> FILE REFERENCE: RB-125-PCT  
 C--> 10 <140> CURRENT APPLICATION NUMBER: US/09/876,796B  
 C--> 11 <141> CURRENT FILING DATE: 2001-08-08  
 13 <150> PRIOR APPLICATION NUMBER: US 60/210,446  
 14 <151> PRIOR FILING DATE: 2000-06-08  
 16 <160> NUMBER OF SEQ ID NOS: 48  
 18 <170> SOFTWARE: Microsoft Word  
 20 <210> SEQ ID NO: 1  
 21 <211> LENGTH: 19  
 22 <212> TYPE: PRT  
 23 <213> ORGANISM: Tenebrio molitor  
 25 <223> OTHER INFORMATION: N-terminal sequence of protein Tm 12.86  
 27 <400> SEQUENCE: 1  
 28 Leu Thr Asp Glu Gln Ile Gln Lys Arg Asn Lys Ile Ser Lys Glu Cys  
 29 1 5 10 15  
 31 Gln Gln Val  
 32 19  
 34 <210> SEQ ID NO: 2  
 35 <211> LENGTH: 576  
 36 <212> TYPE: DNA  
 37 <213> ORGANISM: Tenebrio molitor  
 39 <223> OTHER INFORMATION: Non-his-tagged, signal plus, Tm 13.17  
 41 <400> SEQUENCE: 2  
 42 gtggatccaa agaattcggc acgagactac taag atg aag ttg ctc 46  
 43 Met Lys Leu Leu  
 44 -15  
 46 tgt tgt cta atc tcc ctc att ctg ttg gtc aca gtt cag gcc ctg 91  
 47 Cys Cys Leu Ile Ser Leu Ile Leu Leu Val Thr Val Gln Ala Leu  
 48 -10 -5 1  
 50 acc gag gca caa att gag aaa ctg aac aag atc agc aaa aaa tgt 136  
 51 Thr Glu Ala Gln Ile Glu Lys Leu Asn Lys Ile Ser Lys Lys Cys  
 52 5 10 15  
 54 caa aat gaa agt gga gtg tcg caa gag atc ata acc aaa gct cgc 181  
 55 Gln Asn Glu Ser Gly Val Ser Gln Glu Ile Ile Thr Lys Ala Arg  
 56 20 25 30  
 58 aac ggt gac tgg gag gac gat cct aaa ctg aaa cgc caa gtt ttt 226  
 59 Asn Gly Asp Trp Glu Asp Asp Pro Lys Leu Lys Arg Gln Val Phe  
 60 35 40 45  
 62 tgc gtg gcc agg aac gcc ggt ctg gcc acg gaa tcg gga gag gtg 271  
 63 Cys Val Ala Arg Asn Ala Gly Leu Ala Thr Glu Ser Gly Glu Val

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64          50          55          60
66 gtg gtc gac gtg ttg agg gag aag gtg.agg aag gtc act gac aac      316
67 Val Val Asp Val Leu Arg Glu Lys Val Arg Lys Val Thr Asp Asn
68          65          70          75
70 gac gaa gaa act gag aaa atc atc aat aag tgc gcc gtc aag aga      361
71 Asp Glu Glu Thr Glu Lys Ile Ile Asn Lys Cys Ala Val Lys Arg
72          80          85          90
74 gat act gtt gaa gag acg gtg ttc aat act ttc aaa tgt gtc atg      406
75 Asp Thr Val Glu Glu Thr Val Phe Asn Thr Phe Lys Cys Val Met
76          95          100          105
78 aaa aac aag cca aag ttc tca cca gtt gat tga accaccacga      449
79 Lys Asn Lys Pro Lys Phe Ser Pro Val Asp
80          110          115
82 ctagtagatg gttcaaattgg tgtgctttac atataaaaat aaagtgtttc      499
84 tgatgtaaaa aaaaaaaaaa aaaaaaaaaa aactcgagag tattctagag      549
86 cgccgcggg cccatcgttt tccaccc      576
89 <210> SEQ ID NO: 3
90 <211> LENGTH: 134
91 <212> TYPE: PRT
92 <213> ORGANISM: Tenebrio molitor
94 <223> OTHER INFORMATION: Precursor Protein for Tm 13.17
96 <400> SEQUENCE: 3
97 Met Lys Leu Leu Cys Cys Leu Ile Ser Leu Ile Leu Leu Val Thr Val
98          -15          -10          -5
100 Gln Ala Leu Thr Glu Ala Gln Ile Glu Lys Leu Asn Lys Ile Ser Lys
101          1          5          10
103 Lys Cys Gln Asn Glu Ser Gly Val Ser Gln Glu Ile Ile Thr Lys Ala
104 15          20          25          30
106 Arg Asn Gly Asp Trp Glu Asp Asp Pro Lys Leu Lys Arg Gln Val Phe
107          35          40          45
109 Cys Val Ala Arg Asn Ala Gly Leu Ala Thr Glu Ser Gly Glu Val Val
110          50          55          60
112 Val Asp Val Leu Arg Glu Lys Val Arg Lys Val Thr Asp Asn Asp Glu
113          65          70          75
115 Glu Thr Glu Lys Ile Ile Asn Lys Cys Ala Val Lys Arg Asp Thr Val
116          80          85          90
118 Glu Glu Thr Val Phe Asn Thr Phe Lys Cys Val Met Lys Asn Lys Pro
119 95          100          105          110
121 Lys Phe Ser Pro Val Asp
122          115
125 <210> SEQ ID NO: 4
126 <211> LENGTH: 116
127 <212> TYPE: PRT
128 <213> ORGANISM: Tenebrio molitor
130 <223> OTHER INFORMATION: Mature Protein for Tm 13.17
132 <400> SEQUENCE: 4
133 Leu Thr Glu Ala Gln Ile Glu Lys Leu Asn Lys Ile Ser Lys Lys Cys
134 1          5          10          15
136 Gln Asn Glu Ser Gly Val Ser Gln Glu Ile Ile Thr Lys Ala Arg Asn

```

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137          20          25          30
139 Gly Asp Trp Glu Asp Asp Pro Lys Leu Lys Arg Gln Val Phe Cys Val
140          35          40          45
142 Ala Arg Asn Ala Gly Leu Ala Thr Glu Ser Gly Glu Val Val Val Asp
143          50          55          60
145 Val Leu Arg Glu Lys Val Arg Lys Val Thr Asp Asn Asp Glu Glu Thr
146 65          70          75          80
148 Glu Lys Ile Ile Asn Lys Cys Ala Val Lys Arg Asp Thr Val Glu Glu
149          85          90          95
151 Thr Val Phe Asn Thr Phe Lys Cys Val Met Lys Asn Lys Pro Lys Phe
152          100          105          110
154 Ser Pro Val Asp
155          115
158 <210> SEQ ID NO: 5
159 <211> LENGTH: 481
160 <212> TYPE: DNA
161 <213> ORGANISM: Tenebrio molitor
163 <223> OTHER INFORMATION: Non-His-tagged, Signal plus, Clone 2.2
165 <400> SEQUENCE: 5
166 ggcacgagca aaa atg aaa ctc ctc ttg tgc ttt gcg ttc gcc gcc      46
167          Met Lys Leu Leu Leu Cys Phe Ala Phe Ala Ala
168          -15          -10
170 atc gtc atc gga gct cag gct ctc acc gac gaa cag ata cag aaa      91
171 Ile Val Ile Gly Ala Gln Ala Leu Thr Asp Glu Gln Ile Gln Lys
172          -5          1          5
174 agg aac aag atc agc aaa gaa tgc cag cag gtg tcc gga gtg tcc      136
175 Arg Asn Lys Ile Ser Lys Glu Cys Gln Gln Val Ser Gly Val Ser
176          10          15          20
178 caa gag acg atc gac aaa gtc cgc aca ggt gtc ttg gtc gat gat      181
179 Gln Glu Thr Ile Asp Lys Val Arg Thr Gly Val Leu Val Asp Asp
180          25          30          35
182 ccc aaa atg aag aag cac gtc ctc tgc ttc tcg aag aaa act gga      226
183 Pro Lys Met Lys Lys His Val Leu Cys Phe Ser Lys Lys Thr Gly
184          40          45          50
186 gtg gca acc gaa gcc gga gac acc aat gtg gag gta ctc aaa gcc      271
187 Val Ala Thr Glu Ala Gly Asp Thr Asn Val Glu Val Leu Lys Ala
188          55          60          65
190 aag ctg aag cat gtg gcc agc gac gaa gag gtg gac aag atc gtg      316
191 Lys Leu Lys His Val Ala Ser Asp Glu Glu Val Asp Lys Ile Val
192          70          75          80
194 cag aag tgc gtg gtc aag aag gcc aca cca gag gaa acg gct tat      361
195 Gln Lys Cys Val Val Lys Lys Ala Thr Pro Glu Glu Thr Ala Tyr
196          85          90          95
198 gac acc ttc aag tgt att tac gac agt aaa cct gat ttc tct cct      406
199 Asp Thr Phe Lys Cys Ile Tyr Asp Ser Lys Pro Asp Phe Ser Pro
200          100          105          110
202 att gat taa ttgttttgta ttgactgaa ttttgacaat aaaggtaata      455
203 Ile Asp
204          115

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## RAW SEQUENCE LISTING

DATE: 02/28/2002

PATENT APPLICATION: US/09/876,796B

TIME: 12:22:24

Input Set : A:\Rbl25seq.txt

Output Set: N:\CRF3\02282002\I876796B.raw

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206 tcgttatgta aaaaaaaaaa aaaaaa                                481
209 <210> SEQ ID NO: 6
210 <211> LENGTH: 482
211 <212> TYPE: DNA
212 <213> ORGANISM: Tenebrio molitor
214 <223> OTHER INFORMATION: Non-His-tagged, Signal plus, Clone 2.3
216 <400> SEQUENCE: 6
217 ggcacgagca aaa atg aaa ctc ctc ttg tgc ttt gct ttc gcc gcc      46
218             Met Lys Leu Leu Leu Cys Phe Ala Phe Ala Ala
219             -15                               -10
221 atc gtc atc gga gct cag gct ctc acc gac gaa cag ata cag aaa      91
222 Ile Val Ile Gly Ala Gln Ala Leu Thr Asp Glu Gln Ile Gln Lys
223             -5                               1                               5
225 agg aac aag atc agc aaa gaa tgc cag cag gtg tcc gga gtg tcc      136
226 Arg Asn Lys Ile Ser Lys Glu Cys Gln Gln Val Ser Gly Val Ser
227             10                               15                               20
229 caa gag acg atc gac aaa gtc cgc aca ggt gtc ttg gtc gac gat      181
230 Gln Glu Thr Ile Asp Lys Val Arg Thr Gly Val Leu Val Asp Asp
231             25                               30                               35
233 ccc aaa atg aag aag cac gtc ctc tgc ttc tcg aag aaa act gga      226
234 Pro Lys Met Lys Lys His Val Leu Cys Phe Ser Lys Lys Thr Gly
235             40                               45                               50
237 gtg gca acc gaa gcc gga gac acc aat gtg gag gta ctc aaa gcc      271
238 Val Ala Thr Glu Ala Gly Asp Thr Asn Val Glu Val Leu Lys Ala
239             55                               60                               65
241 aag ctg aag cat gtg gcc agc gac gaa gaa gtg gac aag atc gtg      316
242 Lys Leu Lys His Val Ala Ser Asp Glu Glu Val Asp Lys Ile Val
243             70                               75                               80
245 cag aag tgc gtg gtc aag aag gcc aca cca gag gaa acg gct tat      361
246 Gln Lys Cys Val Val Lys Lys Ala Thr Pro Glu Glu Thr Ala Tyr
247             85                               90                               95
249 gac acc ttc aag tgt att tac gac agt aaa cct gat ttc tct cct      406
250 Asp Thr Phe Lys Cys Ile Tyr Asp Ser Lys Pro Asp Phe Ser Pro
251             100                               105                               110
252 att gat taa ttgttttgta ttgactgaa ttttgacaat aaaggtacta      455
253 Ile Asp
254             115
256 tcgttatgaa aaaaaaaaaa aaaaaa                                482
259 <210> SEQ ID NO: 7
260 <211> LENGTH: 133
261 <212> TYPE: PRT
262 <213> ORGANISM: Tenebrio molitor
264 <223> OTHER INFORMATION: Precursor Protein for Tm 12.84, Clones 2.2, 2.3, and 7.5
266 <400> SEQUENCE: 7
267 Met Lys Leu Leu Leu Cys Phe Ala Phe Ala Ala Ile Val Ile Gly Ala
268             -15                               -10                               -5
270 Gln Ala Leu Thr Asp Glu Gln Ile Gln Lys Arg Asn Lys Ile Ser Lys
271             1                               5                               10
273 Glu Cys Gln Gln Val Ser Gly Val Ser Gln Glu Thr Ile Asp Lys Val

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## RAW SEQUENCE LISTING

DATE: 02/28/2002

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Output Set: N:\CRF3\02282002\I876796B.raw

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274 15          20          25          30
276 Arg Thr Gly Val Leu Val Asp Asp Pro Lys Met Lys Lys His Val Leu
277          35          40          45
279 Cys Phe Ser Lys Lys Thr Gly Val Ala Thr Glu Ala Gly Asp Thr Asn
280          50          55          60
282 Val Glu Val Leu Lys Ala Lys Leu Lys His Val Ala Ser Asp Glu Glu
283          65          70          75
285 Val Asp Lys Ile Val Gln Lys Cys Val Val Lys Lys Ala Thr Pro Glu
286          80          85          90
288 Glu Thr Ala Tyr Asp Thr Phe Lys Cys Ile Tyr Asp Ser Lys Pro Asp
289 95          100          105          110
291 Phe Ser Pro Ile Asp
292          115
295 <210> SEQ ID NO: 8
296 <211> LENGTH: 115
297 <212> TYPE: PRT
298 <213> ORGANISM: Tenebrio molitor
300 <223> OTHER INFORMATION: Mature Protein for Tm 12.84, Clones 2.2, 2.3, and 7.5
302 <400> SEQUENCE: 8
303 Leu Thr Asp Glu Gln Ile Gln Lys Arg Asn Lys Ile Ser Lys Glu Cys
304 1          5          10          15
306 Gln Gln Val Ser Gly Val Ser Gln Glu Thr Ile Asp Lys Val Arg Thr
307          20          25          30
309 Gly Val Leu Val Asp Asp Pro Lys Met Lys Lys His Val Leu Cys Phe
310          35          40          45
312 Ser Lys Lys Thr Gly Val Ala Thr Glu Ala Gly Asp Thr Asn Val Glu
313          50          55          60
315 Val Leu Lys Ala Lys Leu Lys His Val Ala Ser Asp Glu Glu Val Asp
316 65          70          75          80
318 Lys Ile Val Gln Lys Cys Val Val Lys Lys Ala Thr Pro Glu Glu Thr
319          85          90          95
321 Ala Tyr Asp Thr Phe Lys Cys Ile Tyr Asp Ser Lys Pro Asp Phe Ser
322          100          105          110
324 Pro Ile Asp
325          115
328 <210> SEQ ID NO: 9
329 <211> LENGTH: 481
330 <212> TYPE: DNA
331 <213> ORGANISM: Tenebrio molitor
333 <223> OTHER INFORMATION: Non-His-tagged, Signal plus, Clone 3.4
335 <400> SEQUENCE: 9
336 ggcacgagca aaa atg aaa ctc ctc ttg tgc ttt gct ttc gcc gcc          46
337          Met Lys Leu Leu Leu Cys Phe Ala Phe Ala Ala
338          -15          -10
340 atc gtc atc gga gct cag gct ctc acc gac gaa cag ata cag aaa          91
341 Ile Val Ile Gly Ala Gln Ala Leu Thr Asp Glu Gln Ile Gln Lys
342          -5          1          5
344 agg aac aag atc agc aaa gaa tgc cag cag gtg tcc gga gtg tcc          136
345 Arg Asn Lys Ile Ser Lys Glu Cys Gln Gln Val Ser Gly Val Ser

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Use of n and / or Xaa has been detected in the Sequence Listing. Review the Sequence Listing to ensure a corresponding explanation is present in the <220> to <223> fields of each sequence using n or Xaa.

## VERIFICATION SUMMARY

DATE: 02/28/2002

PATENT APPLICATION: US/09/876,796B

TIME: 12:22:25

Input Set : A:\Rbl25seq.txt

Output Set: N:\CRF3\02282002\I876796B.raw

L:10 M:270 C: Current Application Number differs, Replaced Current Application Number

L:11 M:271 C: Current Filing Date differs, Replaced Current Filing Date

L:1880 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:44

L:1888 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:44

L:1889 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:44

L:1912 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:44

L:1913 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:44

L:1916 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:44

L:1920 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:44

L:1934 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:45

L:1935 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:45

L:1938 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:45

L:1939 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:45

L:1942 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:45

L:1946 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:45

L:1950 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:45

L:1954 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:45

L:1958 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:45

L:1962 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:45

L:1966 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:45

L:1970 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:45

L:1974 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:45

L:1989 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:46

L:1993 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:46

L:1997 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:46

L:2001 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:46

L:2005 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:46

L:2009 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:46

L:2013 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:46

L:2017 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:46

L:2021 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:46

L:2025 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:46

L:2029 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:46

L:2044 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:47

L:2048 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:47

L:2052 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:47

L:2056 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:47

L:2060 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:47

L:2064 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:47

L:2068 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:47

L:2072 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:47

L:2076 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:47

L:2080 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:47

L:2084 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:47

L:2099 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:48

L:2102 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:48

L:2105 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:48

L:2108 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:48

## VERIFICATION SUMMARY

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Input Set : A:\Rb125seq.txt

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L:2111 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:48  
L:2114 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:48  
L:2117 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:48  
L:2120 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:48